Grey Matter Pathology in Multiple Sclerosis

Marco Vercellino, MD, Federica Plano, MD, Barbara Votta, Roberto Mutani, MD, Maria Teresa Giordana, MD, PhD, and Paola Cavalla, MD

Abstract
The aim of our study is to evaluate the extent and distribution of grey matter demyelinating lesions in multiple sclerosis (MS), addressing also neuronal loss and synaptic loss. Whole coronal sections of 6 MS brains and 6 control brains were selected. Immunohistochemistry was performed for myelin basic protein, neurofilaments, synaptophysin, ubiquitin, and activated caspase-3. Neuronal density and optical density of synaptophysin staining were estimated in cortical lesions and compared with those observed in corresponding areas of normal (i.e., nondemyelinated) cortex in the same section. Demyelinating lesions were observed in the cerebral cortex, in the thalamus, basal ganglia, and in the hippocampus. The percentage of demyelinated cortex was remarkable in 2 cases of secondary progressive MS (48% and 25.5%, respectively). Neuronal density was significantly reduced in cortical lesions (18–23% reduction), if compared with adjacent normal cortex, in the 2 cases showing the higher extent of cortical demyelination; in the same cases, very rare apoptotic neurons expressing caspase-3 were observed in cortical lesions and not in adjacent normal cortex. No significant decrease in optical density of synaptophysin staining was observed in cortical lesions and not in adjacent normal cortex. Grey matter demyelination and neuronal loss could contribute to disability and cognitive dysfunctions in MS.

Key Words: Apoptosis, Cortical, Demyelination, Grey matter, Multiple sclerosis, Neuronal density, Synaptic density.

INTRODUCTION
Although multiple sclerosis (MS) is still widely regarded primarily as a white matter disease, the sporadic occurrence of demyelinating lesions in grey matter has been described in earlier neuropathologic studies (1, 2). However, the frequency of demyelinating lesions in grey matter in MS is grossly underestimated, both with the conventional magnetic resonance imaging (MRI) techniques and with the common histochemical myelin staining techniques; sensibility for grey matter demyelinating lesions may be improved using immunohistochemistry for myelin proteins (3).

According to the most recent studies (3–5), grey matter involvement appears to be widespread in MS. Because previous neuropathologic studies were performed examining only a limited portion of brain tissue, a complete picture of the actual extent of grey matter demyelination is lacking.

Recent MRI studies, using techniques such as magnetic transfer ratio (MTR), diffusion tensor imaging (DTI), and MRI spectroscopy, have shown diffuse pathologic alterations in normal-appearing grey matter in patients with MS, already present in the early stages of disease and more prominent in the progressive stage (6–12). The correlation between the extent of normal-appearing grey matter pathology, as observed with MRI, and clinical disability, is still a matter of discussion (7, 10). Cognitive deficits, common in patients with MS, could correlate with the extent of normal-appearing grey matter pathology (9).

Limited data are available on phenomena such as inflammation and neurodegeneration in demyelinated grey matter. Acute transection of axons and dendrites has been described in grey matter lesions (4). Data consistent with a reduction of neuronal or axonal density in the grey matter in patients with MS have been also suggested by MRI spectroscopy studies, showing a reduction of the neuroaxonal marker N-acetyl-aspartate both in the thalamus (13, 14) and in the cortex (15, 16).

In this study, we have analyzed coronal sections from MS brains with the aim of evaluating the extent of grey matter demyelination and the pattern of pathologic alterations in the grey matter, addressing also neuronal and synaptic loss and apoptosis in grey matter demyelinating lesions. Data have been correlated with age, duration of disease, and course of disease.

MATERIALS AND METHODS
This study was performed on formalin-fixed, paraffin-embedded archival material of 6 autopsic MS brains: 3 relapsing–remitting (RR) MS and 3 secondary progressive (SP) MS cases (Table 1). Histochemistry and immunohistochemistry for myelin basic protein was also performed on 6 control brains of patients without neurologic diseases.

Mean duration of the disease course was 14.3 years (median, 11.5 years; range, 8–29 years); mean age of death was 52 years (median, 51 years; range, 39–66 years). All patients showed significant disability, being unable to walk without assistance or wheelchair-bound (Expanded Disability Status Scale score [EDSS] ≥ 6). The cases were defined as having RR MS or SP MS retrospectively from hospital records. RR MS was defined by the presence of relapses and absence of recorded progression of disability between relapses. SP MS was defined by progression of disability independently of relapses, after a RR MS phase (17).
sections were treated with 3% H\textsubscript{2}O\textsubscript{2} for 10 minutes and then primary antibodies in control sections. After deparaffinization, consecutive sections using an avidin-biotin method, omitting the incubation of each case was therefore obtained.

perform immunohistochemical techniques; an extensive exam-

10-

through the thalamus and the basal ganglia. Whole coronal

sections were stained with hematoxylin and eosin, Luxol fast blue myelin stain, and Heidenhain’s hematoxylin.

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TABLE 1. Clinical Features of the Patients with Multiple Sclerosis (MS) Included in the Study

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Gender</th>
<th>Age at Death</th>
<th>Duration of Disease</th>
<th>Disease Course</th>
<th>Cause of Death</th>
<th>EDSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>39</td>
<td>13</td>
<td>RR</td>
<td>Status epilepticus</td>
<td>6.0</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>40</td>
<td>10</td>
<td>RR</td>
<td>Breast cancer</td>
<td>6.0</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>44</td>
<td>8</td>
<td>RR</td>
<td>Spinal glioma</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>58</td>
<td>16</td>
<td>SP</td>
<td>Intestinal occlusion</td>
<td>8.0</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>65</td>
<td>29</td>
<td>SP</td>
<td>Decubitus infection</td>
<td>9.0</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>66</td>
<td>10</td>
<td>SP</td>
<td>Pneumonia</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Histochemistry

A coronal section of each brain was obtained cutting through the thalamus and the basal ganglia. Whole coronal 10-\mu m sections were stained with hematoxylin and eosin, Luxol fast blue myelin stain, and Heidenhain’s hematoxylin stain.

Immunohistochemistry

Each coronal section was dissected in smaller blocks to perform immunohistochemical techniques; an extensive examination of each case was therefore obtained.

Immunohistochemistry was performed on 5-\mu m consecutive sections using an avidin-biotin method, omitting the primary antibodies in control sections. After deparaffinization, sections were treated with 3% H\textsubscript{2}O\textsubscript{2} for 10 minutes and then heated by microwave at subboiling temperature for 15 minutes in citrate buffer, pH 6.0. The sections were incubated with 10% normal serum for 30 minutes to block nonspecific staining; they were later incubated overnight at 4°C with the primary antibodies listed in Table 2. After washing with TBS, the sections were incubated at room temperature for 30 minutes with peroxidase-conjugated secondary antibodies (Dako) followed by incubation with the ABC complex (Dako) for 30 minutes. Peroxidase labeling was visualized with 10% 3,3-diaminobenzidine. Tissue sections were counterstained with hematoxylin.

The following antibodies were used: antimyelin basic protein (MBP), antineurofilament, antiuibiquitin, antisyaptophysin, and anticleaved caspase-3 (Table 2).

DNA fragmentation in cell nuclei was determined with the method of in situ hybridization or terminal dUTP nick-end labeling (TUNEL) to identify apoptotic nuclei (18, 19). After deparaffinization, sections were incubated with proteinase K (0.5–2%) and then treated with 3% H\textsubscript{2}O\textsubscript{2} for 10 minutes. The sections were incubated with TdT 20 U (Boehringer, Mannheim, Germany) and dUTP 1 nM (Boehringer) in TdT buffer for 120 minutes at 37°C. After washing with 2× SSC 300 nM and with TBS, the sections were incubated at 37°C for 30 minutes with peroxidase-conjugated antifluorescein antibodies (Boehringer). Peroxidase labeling was visualized with 10% 3,3-diaminobenzidine. Tissue sections were counterstained with hematoxylin.

Measurement of the Extent of Demyelination

The MBP-stained sections were examined using a Zeiss Axiospho microscope. Images were acquired using a Nikon Digital Sight DS-DM camera. The area of demyelination and the total area of white and grey matter in each MBP-stained section were measured on the digital image with the Eclipse.Net software, version 1.16.6 (Laboratory Imaging). Values were compounded and the percentages of demyelinated cortex and white matter were calculated for each case.

Statistical analysis for correlations between extension of grey matter demyelination and parameters such as age and duration of disease were made with the Spearman rank correlation test. Comparisons of the extension of grey matter demyelination between different brain regions and between RR and SP cases and comparisons of the extension of white and grey matter demyelination were performed using the Mann-Whitney U test. SPSS software was used for statistical analysis.

Lesion Classification

Cortical demyelinating lesions were classified according to Bø et al. (3): type I lesions extended across both white and grey matter (leukocortical lesions); type II lesions were located within the cortex and did not extend on the surface of the brain or to the subcortical white matter; type III lesions extended from the surface of the brain into the cortex, sparing the deeper layers of the cortex (subpial lesions); and type IV lesions extended throughout the full width of the cortex but did not reach into the subcortical white matter.

Evaluation of Synaptic Density

Synaptic density was evaluated measuring optical density of synaptophysin staining according to previously published methods (20–22). The analysis was performed using Scion Image software (version 4.0.2; National Institutes of Health). Optical density measured in the cortical lesions was compared with optical density measured in corresponding areas of adjacent normal (i.e. nondemyelinated) cortex in the same cortical layers. The values of optical density obtained in the demyelinated cortex and in the normal cortex were compared using a 2-tailed Student t-test. At least 6 lesions were evaluated for each coronal section.

Estimation of Neuronal Density

Cortical demyelinating lesions were determined in MBP-immunostained sections and then identified on 5-\mu m Nissl-stained consecutive sections to count neurons in cortical lesions.

Images of demyelinated and normal (i.e. nondemyelinated) cortex were acquired from the Nissl-stained sections with a digital camera in 20× microscopic fields. Neurons were counted both in normal and demyelinated cortex, examining...
the images on the computer video display unit by an observer (MV) blinded to demyelinated cortex/normal cortex status. Neurons were counted only when the nucleolus was clearly visible. The number of neurons in each microscopic field was adjusted with the Abercrombie method (23) and expressed as neuronal density (neurons/mm²).

The neuronal density in each cortical lesion was compared with the neuronal density in an adjacent area of normal cortex in the same section in the corresponding cortical layers. Ten microscopic fields were evaluated for each lesion and for each corresponding area of normal cortex. At least 6 lesions were evaluated for each coronal section. The values of neuronal density obtained in the demyelinated cortex and in the normal cortex were compared using a 2-tailed Student t-test.

RESULTS

White Matter Demyelinating Lesions

Several partially confluent demyelinating lesions with relative axon-sparing and glial scarring were observed in the white matter in all cases. Active lesions, with macrophages containing Luxol fast blue-positive material, were identified in 2 cases of RR MS. The extent of white matter demyelination ranged from 4% to 75.5% of the total white matter area in the coronal section (mean, 21.75%; median, 11%) (Table 3).

Grey Matter Demyelinating Lesions

Histochemical methods (Luxol fast blue myelin stain and Heidenhain’s hematoxylin stain) detected rare and ill-defined grey matter lesions in the cortex and in the deep grey nuclei. Anti-MBP immunostaining allowed precise and reliable identification of demyelinating lesions in the grey matter. Several demyelinating lesions were identified in the cortex in all cases (Table 3; Fig. 1A–F). Most lesions (> 90%) were limited to the more superficial layers of the cerebral cortex (layer I to layer III–IV), often extending for very long stretches and on several gyri (type III lesions) (Fig. 2B, D, E). A few small lesions within the width of the cortex were observed (type II lesions), as well as a few lesions including the whole width of the cortex up to the border between the cortex and the white matter (type IV lesions) (Fig. 2A). Only one leuкоkortical lesion (type I lesion) was observed. Cortical lesions appeared to be more frequent in the cingulate gyrus (mean percentage of demyelinated cortex 19.4%; Fig. 2A), in the temporal lobe (17.2%; Fig. 2B), and in the insula (Fig. 2D; 16.3%), and less frequent in the frontal lobe (10.9%; Fig. 2E) (Table 4). Such differences, however, were not statistically significant.

Demyelinating lesions were also identified in the dentate gyrus of the hippocampus in one case (Fig. 2C), in the globus pallidus in one case, and in the thalamus in 2 cases. In one case, thalamic demyelination was conspicuous, involving up to one third of the thalamus in the coronal section. The extent of cortical demyelinating lesions ranged from 2% to 48% of the total cortical area in the coronal section (mean, 14.8%; median, 3.25%). In 2 cases of SP MS, the extent of cortical demyelination was remarkable, displaying a pattern of generalized subpial demyelination (Table 3; Figs. 1E, F, and 2B, D, E).

No correlation was found in the extent of demyelination between white matter and grey matter. The extent of grey matter demyelination showed a positive trend with age and duration of disease and was much higher in cases of SP MS than RR MS. Such differences, however, were not statistically significant.

No inflammation or gliosis were observed in the grey matter demyelinating lesions. Antineurofilament staining demonstrated a relative sparing of axons in grey matter demyelinating lesions. No demyelinating lesion in white or grey matter was observed in control brains.

Neuronal Density

Neuronal density was significantly reduced in cortical lesions when compared with adjacent normal cortex in the 2 cases (case nos. 5 and 6) showing the highest extent of cortical demyelination (25.5% and 48%) (demyelinated cortex: mean, 54.6 neurons/mm² and 50 neurons/mm², respectively; normal cortex: mean, 66.5 neurons/mm² and 64.9 neurons/mm², respectively) (p < 0.005; Student t-test). In the remaining cases, a moderate reduction of neuronal density in demyelinated cortex was observed when compared with adjacent normal cortex; this difference, however, was not statistically significant (Table 5).

Synaptic Density

No significant differences in optical density of synaptophysin staining were observed between demyelinated cortex and adjacent normal cortex in any of the 6 cases.

Apoptosis

Immunohistochemistry for activated caspase-3 was used for the detection of the apoptotic process. The TUNEL technique did not yield satisfying results in this autopsic series, proving to be nonspecific and unreliable. Small nuclei stained with anticaspase-3 antibody were rarely found in demyelinated cortex and in normal cortex (i.e. nondemyelinated), possibly belonging to oligodendrocytes. In the 2 cases of SP MS with the higher extent of cortical demyelination (patients 5 and 6), very rare neuronal nuclei immunostained with caspase-3 were identified within cortical lesions (approximately 0.39 and 0.48 nuclei/100 mm², respectively) (Fig. 2F). No caspase-3-stained neuronal nuclei were observed in the normal cortex, thalamus, or basal ganglia.

TABLE 3. Correlations Between Clinical and Pathologic Features

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Percentage of Demyelinated Cortex</th>
<th>Percentage of Demyelinated White Matter</th>
<th>Age</th>
<th>Duration of Disease</th>
<th>Disease Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5%</td>
<td>75.5%</td>
<td>39</td>
<td>13</td>
<td>RR</td>
</tr>
<tr>
<td>2</td>
<td>2%</td>
<td>8.5%</td>
<td>40</td>
<td>10</td>
<td>RR</td>
</tr>
<tr>
<td>3</td>
<td>3.5%</td>
<td>4%</td>
<td>44</td>
<td>8</td>
<td>RR</td>
</tr>
<tr>
<td>4</td>
<td>3%</td>
<td>13.5%</td>
<td>58</td>
<td>16</td>
<td>SP</td>
</tr>
<tr>
<td>5</td>
<td>48%</td>
<td>21.5%</td>
<td>65</td>
<td>29</td>
<td>SP</td>
</tr>
<tr>
<td>6</td>
<td>25.5%</td>
<td>7.5%</td>
<td>66</td>
<td>10</td>
<td>SP</td>
</tr>
<tr>
<td>Mean</td>
<td>14.8%</td>
<td>21.75%</td>
<td>52</td>
<td>14.3</td>
<td></td>
</tr>
</tbody>
</table>
Ubiquitin Staining

In all cases, a diffuse granular ubiquitin staining was observed in the white matter, stopping at the edge of the lesions, together with the absence of myelin; no staining was found within the lesions. Such pattern was not observed in cortical demyelinating lesions. A diffuse granular ubiquitin staining was observed both in normal and demyelinated grey matter, even if the density of staining was lower if compared with white matter.

DISCUSSION

This study confirms the importance of grey matter demyelination in MS, especially in cases with a long duration of disease. Our extensive examination of grey matter pathology in MS, examining whole coronal sections, provides data on the extent and distribution of grey matter demyelination.

Traditional myelin stains such as Luxol fast blue show very low sensitivity for grey matter lesions because the staining of cortical myelin is poor and therefore the contrast between normal cortex and demyelinated cortex is insufficient for a reliable detection. Other myelin stains such as Heidenhain’s hematoxylin show improved sensitivity, but are still considerably inferior to immunohistochemical techniques.

Immunohistochemical techniques, using antibodies targeting MBP or proteolipid protein, allow reliable identification of grey matter demyelination. We confirm the superior reliability of immunostaining techniques, especially regarding purely cortical lesions.

Most cortical lesions observed in our study are subpial lesions, involving the superficial layers of the cortex for several adjacent gyri; such lesions are generally not detectable on conventional MRI scans. In the 2 cases with SP MS and longer duration of disease, almost the full extent of the outer layer of the cortex is demyelinated, a pattern described as generalized subpial demyelination. Demyelination in the deep grey matter is present in 3 cases, especially in the thalamus.

The extent of cortical demyelination is very variable: a trend for a higher extent of cortical demyelination in cases with SP MS and longer duration of disease has been observed in our study, probably not reaching statistical significance because of the limited number of cases examined. This might indicate widespread cortical demyelination as a late event in MS, possibly related to severe disability.

Cortical lesions appear to be frequent in the cingulate gyrus, in the temporal lobe, and in the insula. These data are in

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Percentage of Demyelinated Cortex in Each Region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cingulate Gyrus</td>
</tr>
<tr>
<td>1</td>
<td>20.6%</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>8.4%</td>
</tr>
<tr>
<td>4</td>
<td>7.4%</td>
</tr>
<tr>
<td>5</td>
<td>60.2%</td>
</tr>
<tr>
<td>6</td>
<td>19.5%</td>
</tr>
<tr>
<td>Mean</td>
<td>19.4%</td>
</tr>
</tbody>
</table>

FIGURE 1. Coronal brain sections of cases of relapsing–remitting multiple sclerosis (Luxol fast blue myelin stain): patient 1 (A), patient 2 (B), and patient 3 (C). Coronal brain sections of cases of secondary progressive multiple sclerosis (Luxol fast blue myelin stain): patient 4 (D), patient 5 (E), and patient 6 (F). Grey matter lesions were detected using immunohistochemistry for myelin basic protein on smaller sections obtained from the whole coronal section. Lesions were drawn in black on the whole section to illustrate the extent and distribution of grey matter demyelination; such lesions were not detectable with the Luxol stain.
line with the results of a previous study, obtained on a limited cortical sampling, finding a higher frequency of cortical lesions in the cingulate gyrus (3). In our study, a demyelinating lesion has been identified also in the hippocampus. The involvement of the limbic and temporal cortex, as well as the involvement of the hippocampus, could contribute to the pathogenesis of the cognitive and behavioral dysfunctions of MS.

In this study, a decrease of neuronal density has been observed in cortical demyelinating lesions in the cases showing the higher extent of cortical demyelination. The difference in neuronal density between demyelinated cortex and adjacent normal cortex, in these cases, is approximately 20%. A possible relation between cortical demyelination and neuronal loss might be hypothesized. The finding of a significant neuronal loss in the cerebral cortex of patients with MS with extensive cortical demyelination, not previously described in neuropathologic studies, is consistent with the results of the most recent neuroradiologic studies in vivo, showing a decrease of the neuronal/axonal marker N-acetyl-aspartate in the cortex of patients with MS, particularly progressive MS (15, 16). Moreover, a loss of neurons in the mediodorsal nucleus of the thalamus in cases of SP MS has been described in a previous study comparing MS cases with control brains (13).

Very rare neuronal nuclei stained with anticaspase-3 antibody have been observed in cortical demyelinating lesions in the 2 cases showing the highest extent of cortical demyelination; in the same cases, a significant reduction of neuronal density has been observed in the demyelinated cortex when compared with normal cortex. Caspase-3 is one of the main effector proteases in apoptosis and defines an irreversible stage

**TABLE 5. Neuronal Density in Cortical Demyelinating Lesions Compared with Neuronal Density in Adjacent Normal Cortex**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of demyelinated cortex</td>
<td>2.5%</td>
<td>2%</td>
<td>3.5%</td>
<td>3%</td>
<td>48%</td>
<td>25.5%</td>
</tr>
<tr>
<td>Disease course</td>
<td>RR</td>
<td>RR</td>
<td>RR</td>
<td>SP</td>
<td>SP</td>
<td>SP</td>
</tr>
<tr>
<td>Mean neuronal density (neurons/mm³), demyelinated cortex</td>
<td>60.4</td>
<td>62.7</td>
<td>64.3</td>
<td>62.5</td>
<td>50.0</td>
<td>54.6</td>
</tr>
<tr>
<td>Mean neuronal density (neurons/mm³), normal (i.e. nondemyelinated) cortex</td>
<td>61.6</td>
<td>63.7</td>
<td>64.6</td>
<td>66.2</td>
<td>64.9</td>
<td>66.5</td>
</tr>
<tr>
<td>Difference in neuronal density, normal cortex–demyelinated cortex</td>
<td>–1.9%</td>
<td>–1.6%</td>
<td>–0.5%</td>
<td>–5.6%</td>
<td>–23%</td>
<td>–17.9%</td>
</tr>
<tr>
<td>Student t-test</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>p &lt; 0.005</td>
<td>p &lt; 0.005</td>
</tr>
</tbody>
</table>

FIGURE 2. Cortical demyelinating lesion (myelin basic protein [MBP] immunohistochemistry, 2.5×) in the cingulate gyrus (A) (type IV lesion) from a case of relapsing–remitting multiple sclerosis (patient no. 1). Cortical demyelinating lesions (MBP immunohistochemistry, 2.5×) in the temporal lobe (B) (type III lesion), and in the dentate gyrus of the hippocampus (C) from a case of secondary progressive multiple sclerosis (SP MS) (patient no. 5). Cortical demyelinating lesions (MBP immunostaining) in the insula (D) (20×) (type III lesion, border) and in the frontal lobe (E) (40×) (type III lesion, border) from a case of SP MS (patient no. 6). Caspase-3-stained neuronal nucleus in demyelinated cortex (F) (cleaved caspase-3 immunostaining, 100×) from a case of SP MS (patient no. 6).
in the cell death process; immunohistochemistry for activated caspase-3 is both sensitive and highly specific for the detection of apoptosis (25). Our results confirm the findings of Peterson et al, who demonstrated neuronal apoptosis in a series of seven MS cases using the TUNEL technique (4). The lower number of apoptotic neurons identified in our study might be explained as a consequence of the higher specificity of caspase-3 immuno- 

The number of neurons immunostained with caspase-3 observed on the whole is very low; nevertheless, it must be considered that apoptotic neurons are cleared in 24 hours and immunoreactivity for activated caspase-3 is detectable only for a short span of time. In a disease with a very long course such as MS, neuronal apoptosis could play a role over time. The finding of caspase-3-positive neuronal nuclei only in the 

demyelinated cortex and not in the normal grey matter suggests a possible causal relationship between grey matter demyelination and neuronal apoptosis.

According to our data, neuronal loss in MS is, at least in part, the result of local loss in the demyelinated grey matter and not merely a consequence of retrograde neuronal degeneration after axonal damage in white matter lesions. Loss of neurons in the demyelinated cortex occurs possibly, to some extent, through apoptosis.

The causes underlying neuronal apoptosis and neuronal loss in grey matter demyelinating lesions will be further investigated. The immunopathogenesis of grey matter demyelination appears to be quite different from that of white matter demyelination, lacking inflammatory infiltrates and deposition of complement and immunoglobulins (28, 29). Heterogeneity of the pathogenic process in white matter demyelinating lesions in MS has been described (30); grey matter demyelination could also present a similar heterogeneity. The involvement of soluble myelinotoxic factors diffusing from the cerebrospinal fluid (CSF) has been proposed as a putative mechanism in the pathogenesis of the subpial cortical lesions (3). CSF from patients with MS has been shown to induce neuronal apoptosis in vitro with expression of caspase-3 in the apoptotic nuclei; in this model, the induction of apoptosis can be blocked by caspase-3 inhibitors (31–34). Ectopic B-cell follicles with germinal centers have been described in the leptomeninges of SP MS cases (35); they could represent a possible source of soluble factors harmful to myelin, oligodendrocytes, or neurons. To explain neuronal loss in the demyelinated cortex, a lack of neurotrophic factors necessary for neuronal survival resulting from myelin/oligodendrocytes loss could also be advocated (36). Alternatively, evidence is available for a role of chronic excitotoxic damage to neurons in MS and in experimental autoimmune encephalomyelitis (EAE) (37–40). Axonal transection in cortical lesions could contribute to loss of neurons through retrograde degeneration (4).

Synaptic loss as determined by optical density of synaptophysin staining does not appear to be a main feature of cortical lesions. No difference in optical density of synaptophysin staining has been observed between cortical lesions and adjacent normal cortex. A normal synaptic density is found also in areas in which decreased neuronal density is observed. Mechanisms of synaptic remodeling could perhaps compensate the synaptic loss resulting from loss of neurons and axonal transection. A recent study showed that, in EAE, synaptic loss is remarkable during the acute inflammatory stage but appears to recover during remission (41). On the other hand, in amyotrophic lateral sclerosis, a decrease of neuronal density in the motor cortex without a corresponding loss of synaptic terminals has been described (42). The pattern of ubiquitin staining observed in the white matter has been considered indicative of derangement of transport in the demyelinated portion of axons (43). Such a pattern is not detectable in demyelinated grey matter.

In conclusion, grey matter pathology in MS appears to be widespread and extensive, especially in SP MS. A substantial effect of grey matter demyelination on late clinical disability and on cognitive deficits might be hypothesized as a consequence of neuronal loss and of impairment of conduction through demyelinated axons with functional disruption of cortical networks.

The importance of grey matter demyelination is still underestimated, probably because its neuroradiologic demonstration is difficult (24). The widespread cortical demyelination observed in SP MS cases could account for the poor correlation between clinical disability and lesion load in the white matter determined by conventional MRI (44). The pathogenesis of grey matter lesions appears to differ significantly from the pathogenesis of white matter lesions, and it is still to be determined whether the same therapies could equally influence grey and white matter demyelination.

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